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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Foung *et al.*	Examiner:	Wortman, D.
Serial No.:	09/728,720	Art Unit:	1648
Filed:	December 1, 2000		
For:	PREVENTION AND TREATMENT OF HCV INFECTION EMPLOYING ANTIBODIES DIRECTED AGAINST CONFORMATIONAL EPITOPES		

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. 1.132

I, Steven K. H. Foung, declare as follows:

1. I am currently an Associate Professor of Pathology and the Director of Clinical Laboratories at Stanford University Medical Center.

2. I am a joint inventor of the subject matter disclosed and claimed in United States patent application Serial No. 09/430,489 filed October 29, 1999 and entitled "Prevention and Treatment of HCV Infection Employing Antibodies that Inhibit the Interaction of HCV Virions with their Receptor" (the '489 application).

3. I have reviewed and understood United States patent application Serial No. 09/430,489, filed October 29, 1999 and entitled "Prevention and Treatment of HCV Infection Employing Antibodies that Inhibit the Interaction of HCV Virions," and have also reviewed and understood the International Application published under the Patent Cooperation Treaty (PCT) WO 97/40176 by Persson *et al.* (herein referred to as "Persson et al.").

4. This Declaration is presented for the purpose of overcoming a rejection that the pending claims are anticipated by the reference Persson *et al.* (WO 97/40176). The present Declaration is therefore intended to present scientific data that shows that the anti-HCV antibodies disclosed and claimed in the present claims are different from the antibodies of Persson *et al.*

5 The antigen binding portion of an antibody, generally referred to as the Fab fragment, is made up of a heavy chain and a light chain, each having a variable (V) domain and one or more constant (C) domains (see **Exhibit A**). Certain regions in the V domain of the heavy chain (V_H) and light chains (V_L) show remarkable diversity in amino acid sequence. Systematic analysis localizes these hypervariable regions to three segments within V_H and three segments within V_L. These hypervariable regions are called Comlementarity Determining Regions (CDRs) (CDR1, CDR2, and CDR3).

6. A reference by John L. Xu and Mark M. Davis (*Immunity*, July, 2000 Volume13, pages 37-45, herein referred to as "Xu and Davis") (**Exhibit B**), which I have reviewed and understand, shows that the diversity in antigen binding specificity of an antibody is determined by the

diversity of CDR3 of the V domain of the heavy chain (V_H) alone. Xu and Davis created mice constrained to use a single V_H gene (V_H5-51), but full CDR3 diversity to generate their B cell repertoire. Mice were challenged with a variety of protein and hapten antigens and the development of primary and memory immune responses monitored. (See Exhibits "Experimental System," page 37-38 and "Experimental Procedures," page 43.) Figure 2 shows that the mice respond to antigen challenge with a "normal" immune response (see page 39, column 1).

7. The mice of Xu and Davis were also used to generate monoclonal antibodies to a variety of antigens (see page 39, column 1-2 of **Exhibit B**). As shown in Figure 3, cDNAs encoding the monoclonal antibodies were synthesized and amplified by RT-PCR and then cloned and sequenced. All of the hybridoma sequences characterized differed predominantly in the CDR3 region of the heavy chain. Xu and Davis concluded that "the highly diverse CDR3 loops are the key determinant of specificity to antigen recognition" (Abstract).

8. In the Persson et al. reference, four antibodies are disclosed that bind Hepatitis C Virus E2 antigen. The deduced amino acid sequence of the V_H chain of each antibody is shown in Figures 1A-1D (SEQ ID NOs: 1-4). The deduced amino acid sequence of the V_L chain of each antibody is shown in Figures 2A-2D (SEQ ID NOs: 5-8). The CDR 1, CDR2, and CDR3 regions are underlined in each sequence in the Figures.

9. Experiments have been performed in my laboratory, under my direction to obtain the deduced amino acid sequences of the anti-HCV antibodies disclosed and claimed in the present application. As described in a paper by Chan et al. ("V_H1-69 Gene is Preferentially Used by Hepatitis C Virus-associated B Cell Lymphomas and by normal B cells responding to the E2 viral antigen" *Blood*, 15 February 2001 Volume 97, Number 4, herein referred to as "Chan et al.") (**Exhibit C**), peripheral B cells were isolated from an asymptomatic patient who had a high serum neutralization binding titer to the HCV 1b viral genotype and used to generate anti-HCV E2 human hybridomas. The hybridomas were screened for binding HCV E2 proteins. Ten hybridomas were selected, all of which produced a monoclonal antibody that reacted with HCV E2 envelope glycoprotein. Total cellular RNA was isolated from the selected hybridomas and reverse transcribed. The V region genes were amplified using the polymerase chain reaction (PCR) and sequenced on an automated sequencer. The amino acid sequences of V_H and V_L genes of the antibodies CBH-4B, CBH-4D, CBH-4G, CBH-5, CBH-7, CBH-8C, CBH-8E, CBH-11, CBH-2, and CBH-17 are shown in Figure 1A and 1B of Chan et al., herein referred to as "our antibodies." The CDR 1, CDR2, and CDR3 regions are indicated in Figures 1A and 1B.

10. I have entered the deduced amino acid sequences of Persson et al. (SEQ ID NOs: 1-8) and the deduced amino acid sequences of CBH-4B, CBH-4D, CBH-4G, CBH-5, CBH-7, CBH-8C, CBH-8E, CBH-11, and CBH-17 into a computer database and created alignments of each of the Persson et al. sequences with each the sequences of our antibodies (**Exhibit D**). Specifically, each of the V_H chain sequences of Persson et al. (SEQ ID NOs: 1-4) are aligned with each of the V_H chain sequences of our antibodies (pages 1-4 of **Exhibit D**). In addition, each of the V_L chain

sequences of Persson et al. (SEQ ID NOs: 5-8) are aligned with each of the V_L chain sequences of our antibodies (pages 9-12 of **Exhibit D**). Sequences identical to the Persson et al. sequence are indicated by a yellow background and red foreground. Conservative sequences are indicated by light green background and blue foreground. Red indicates the beginning and end of each CDR1, CDR2, and CDR3 region. Additional alignments of the individual CDR regions of the Persson et al. heavy and light chain sequences with the sequences of our antibodies are shown at pages 5-8 and pages 13-16, respectively, of **Exhibit D**.

11. It is clear from the sequence alignments provided in **Exhibit D** that the sequences of the Persson et al. antibodies and the sequences of our antibodies are different. None of the CDR regions (CDR 1, CDR2, or CDR3) of our antibodies contain any sequences that are identical to the Persson et al. antibodies. Alignments reveal no homologies between our antibodies and the antibodies of Persson et al.

12. As one skilled in the art, I conclude that because the sequence of CDR3 determines the antigen binding specificity of antibodies (Xu and Davis, **Exhibit B**) and because the sequences of our antibodies are different from the sequences disclosed in the Persson et al. reference, the antigen binding specificities of our antibodies are distinct from the antigen binding specificities of the antibodies of the Persson et al. reference. Therefore, the antibodies of the present application are distinct from those of Persson et al. in both structure and specificity.

13. I declare that all statements made herein of my own knowledge are true, and that those statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like are made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the '430 application or any patents that may issue thereon.

Respectfully Submitted,

Dr. Steven K. H. Foung
Date: July 11, 2003